Reduction of carcinogenesis by biobased lignin derivatives

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Lignin component of biomass belongs to the most abudant organic polymers in nature. Biosphere contains 3.10^{11} t lignin with an increase 2.10^{10} t per year. The chemical treatment of wood for paper production yields about 50.10^{6} t lignin per year [1]. Large abudances of lignin make it an ideal source of chemicals. In our studies, lignin component of plant biomass, that is not diggested by man, was investigated in vitro with regard to its ability to bind bile acids – products of cholesterol degradation and nitrosamines – well known potential carcinogens as well as to reduce damage of deoxyribonucleic acids (DNA) in human cells induced by mutagenic chemicals for potential medicinal utility.

The binding affinity of lignin samples derived from kraft pulping and hydrolysis of wood and their methylated, acetylated, reduced and condensed derivatives was tested for sodium cholate in two buffers pH 5.4 and 8.0. It was shown that the ability of lignin (10 mg/ml) to bind sodium cholate (100 ?g/ml) varies from 10 to 90 %. It depends on modification method and genetic origin of wood species. The adsorptive effect of condensed beech kraft lignin was comparable with that of cholestyramine – used in the treatment of hypercholesterolemia, when the ratio of lignin to sodium cholate was 100 : 1. These results are interesting in the light of new finding, that bile acids increase colon tumorigenesis [2].

The potential protective role of lignin against carcinogens was investigated by determination of a binding ability of different lignin preparations for N-nitrosamines. The binding capacity of lignin samples was followed by polarographic determination of concentration of N-nitrosodiethylamine (NDA) remaining in solution after addition of lignin preparations at pH 5.4 and 8.0. The data summarized in Table 1 show, that the binding ability of kraft lignin after H_2SO_4-treatment is rather higher in comparison with that of original sample. The molecular characteristics of sample tested indicate that used modification causes substantional decrease of cross-linking density about 73 % and increase of average molecular weight about 22.7 %. Based on the obtained results the effective enhancement of lignin adsorption capacity can be explain by degradation of cross links and linearization of lignin macromolecule resulting in increase specific surface.

Table 1. Lignin samples used in N-nitrosodiethylamine (NDA) – adsorption studies

Lignin sample	Average molecular weight	Cross-linking density	Binding capacity (?mol/g lignin)	
			pH 5.4	pH 8.0
Kraft lignin	6800	0.0281	10	18
Condensed kraft lignin	8800	0.0076	85	80

In contrast with lignin, all tested carbohydrate preparations (microcrystalline cellulose, holocellulose and hemicelluloses) were poor adsorbents for nitrosamines as well as for bile acids. Also in microbial system *Salmonella typhimurium* mutagenicity of IQ (2-amino-3-methylimidazo [4,5-f] quinoline) was strongly reduced by lignin, while cellulose and xylan were ineffective [3].

Oxidative damage to DNA is thought to be an important etiologic factor in the development of chronic diseases such as cancer. In our experiments the antioxidative effect of lignin biopolymer derived from beechwood hydrolysis on DNA in human cells VH10 cells exposed to model DNA – damaging agent, H_2O_2, was investigated. The level of DNA damage (DNA strand breaks) was measured using single cell

gel electrophoresis, i.e. comet assay described by Slamenová et al. [4]. Fig. 1 shows the level of DNA breaks in control and H_2O_2-treated human cells VH10 cells as well as reduction of breaks by lignin sample. The measure of DNA damage was expressed as "% of tail DNA".

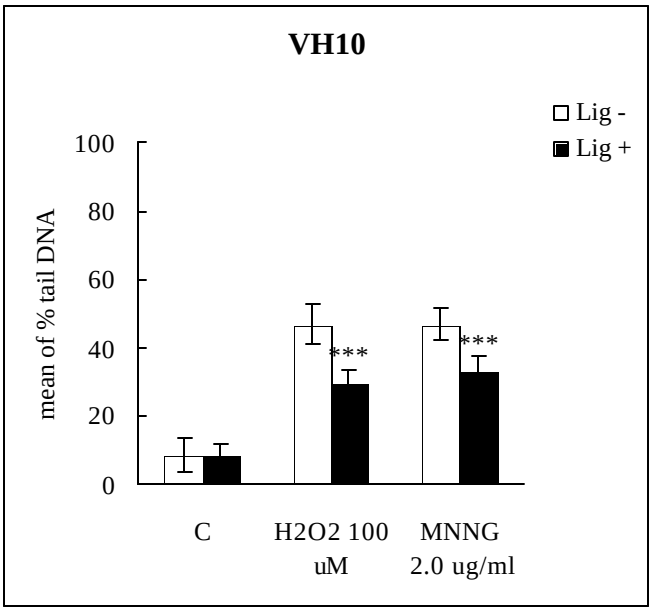

Fig.1. Influence of lignin (50 ? g/ml) on level of DNA breakge induced by H_2O_2 and by MNNG.

It is known, that lignin, which has unique hindered phenolic hydroxyl group, acts as a stabilizer of reactions induced by oxygen and its radical reduction products [5]. Based on this, it can be suggested that phenolic groups of lignin matrix in the presence of oxygen are probably responsible for scavenging of reactive oxygen species and reduction of oxidative DNA damage in lignin pre-treated cells. The comparison of reduction of oxidative damage to DNA by lignin biopolymer and by well-known natural antioxidant vitamin E (? -tocopherol) indicates that lignin was even more effective.

The second series of experiments was performed with N-methyl-N´-nitro-N-nitrosoguanidine (MNNG). In contrast to H_2O_2, MNNG does not induce any significant level of oxidative damage to DNA but it alkylates several positions on DNA. In this case, the presence of lignin in the cultivation medium also significantly reduced single-strand breaks in the contrast to vitamin E that was not effective in cells damaged by the monofunctional alkylating agent MNNG. The reduction of MNNG induced DNA lesions may correlate with the above-described good adsorptive capacity of lignin for N-nitroso compounds.

Conclusion

The revealed antioxidative and adsorptive ability of non-toxic lignin derivatives tested to reduce genotoxic activity of chemicals indicates their prospective application as natural agents for the prevention of carcinogenesis and other diseases instead of those prepared exclusively by organic synthesis.

References

[1] Glasser WG. Forest Prod.J. 31, 1981, p. 24.
[2] Baijal PK, Fitzpatrick DW, Bird RP. Food Chem.Toxicology 36, 1998, p. 601.
[3] Edenharder R, Leopold C, Kries M. Mutat.Res. 341, 1995, p. 303.
[4] Slamenová D, Gábelová A, Ružeková L, Chalupa I, Horváthová E, Farkašová T, Bozsakyová E, Štetina R. Mutat.Res. 383, 1997, p. 243.
[5] Kratzl K, Schaefer W, Claus P, Gratzl J, Schilling P. Monatsh.Chem. 98, 1967, p. 891.